Evaluation of Diffusion in The Dual-Hydrogel System

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INTRODUCTION

Prevascularization is a promising approach to generate vascularization in engineered tissue constructs before transplantation to improve the success of engraftment and minimize ischemia. In order to create the prevascularized tissue construct, we previously developed a perfusable tissue graft consisting of a rigid PCL-TCP scaffold and cell-laden channel containing dual layer hydrogel. This graft has a great potential to accelerate in vitro vascularization by using two different photo-crosslinkable hydrogels such as polyetheleneglycol (PEG) and gelatin-methacryloyl (GelMA). The high mechanical stiffness and slow degradation rate of PEG provides the long-term integrity of the perfusable channel. While the GelMA provides a fast endothelial cell (EC) sprouting environment to accelerate microvascular formation due to its soft and fast degradable properties. In this study, we aimed to build a perfusable model system for in vitro vascularization that can be controlled and maintained by combining the two different hydrogel properties. We first studied the structural, mechanical, and swelling and degradation behaviors of PEG and GelMA. Second, our in vitro model system consisting of a PEG-based channel and angiogenic sprouting GelMA environment was fabricated based on the properties of the hydrogels. Diffusion profiles were tested using Dextran Texas Red solution under microscope and then analyzed using MATLAB.

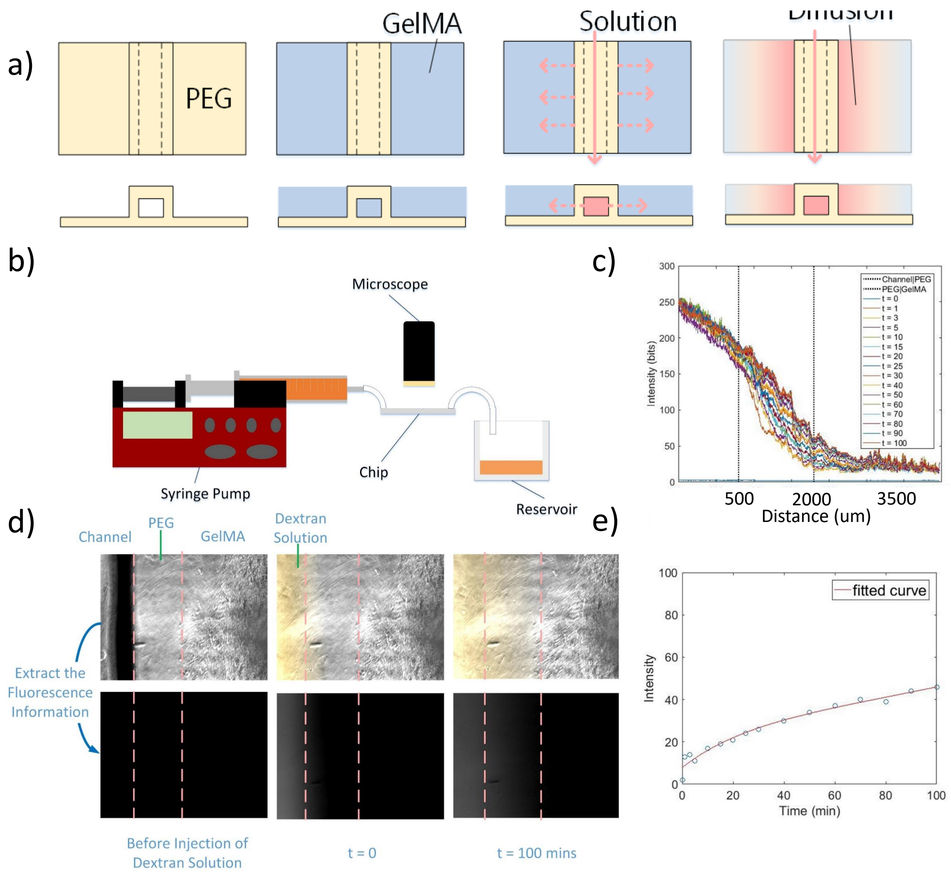

Figure 1. *The Schematic of Device and The Experimental Setup for the Diffusion Test. (a) The process of manufacturing the diffusion device, which includes a PEG channel and a GelMA environment. (b) The experimental setup of the diffusion test. (c) The images captured by the microscope were further analyzed in MATLAB. (d) The bright field and the fluorescence images before the injection of dextran ($t = 0^-$), right after the injection ($t = 0^+$), and after perfusing 100 minutes. (e) The changing dextran intensity with time can be analyzed by the image processing functions in MATLAB.*

METHODS & RESULTS

Two conditions of GelMA and one condition of PEG were used to test regarding porosity, mechanical stiffness and degradation rate of hydrogels. The 10 wt% PEG with exposure time 2 mins, 10 wt% GelMA with exposure time 1.5 (**G-1.5**) and 2.5 (**G-2.5**) mins were used for the diffusion test. The photoinitiator 0.1 wt% lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) was synthesized in our lab, and 0.1 wt% Dextran Texas Red(Sigma Aldrich) 40 kDa was used for the quantification of the diffusion.

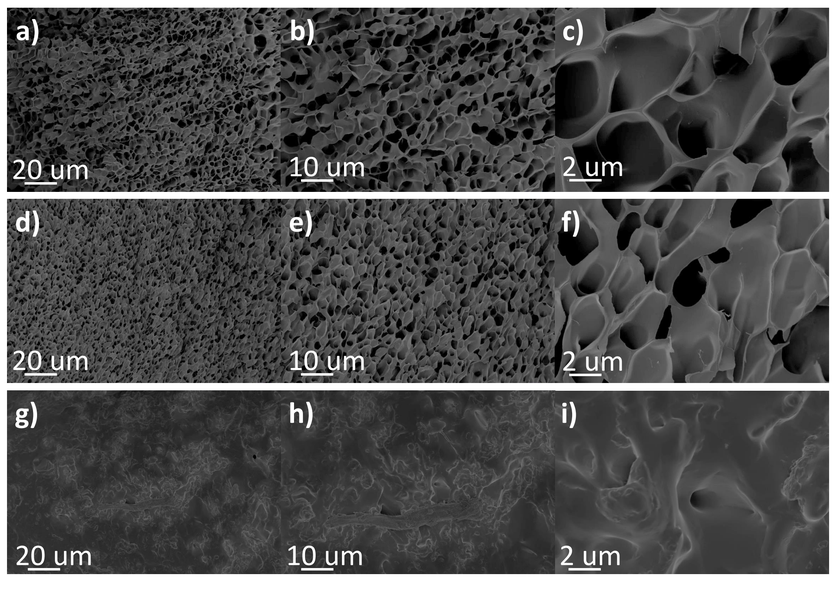

Figure 2. *SEM Images of PEG and GelMA . (a)-(c) GelMA 10% with exposure time 1 minute 30 seconds. (d)-(f) GelMA 10% with exposure time 2 minute 30 seconds. (g)-(i) PEG 10% with exposure time 2 seconds. The pore sizes of G-1.5 and G-2.5 were around 4 and 2 um, respectively. The pores of PEG cannot be observed in the SEM images*

METHODS & RESULTS

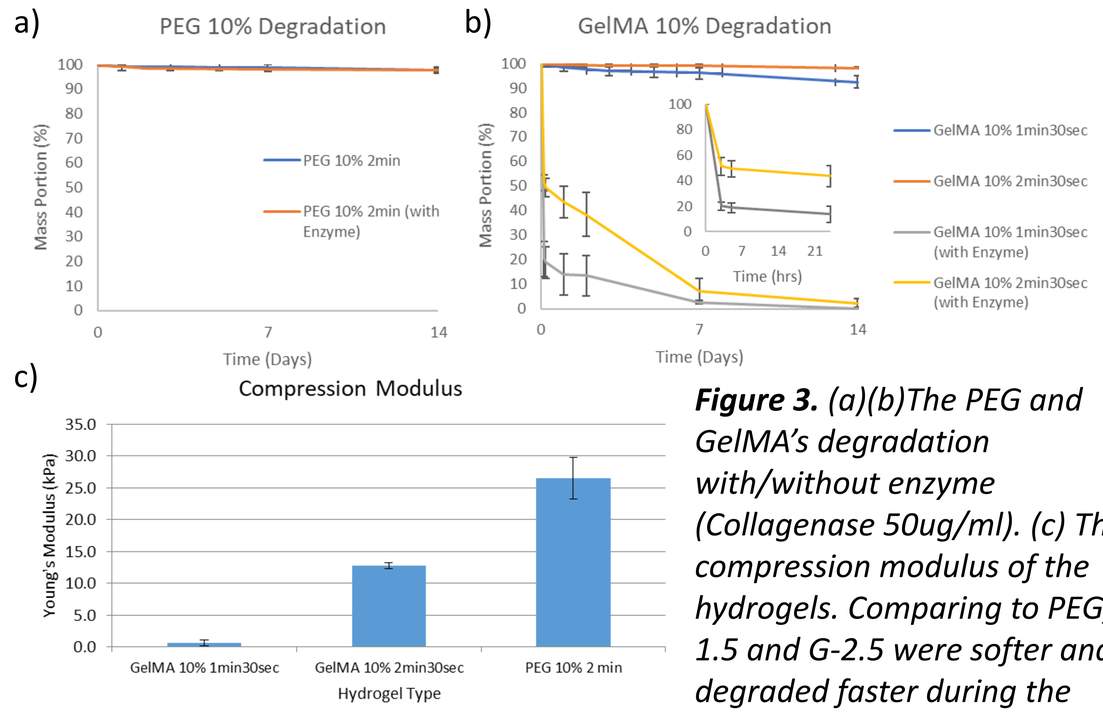

Figure 3. *(a)(b)The PEG and GelMA's degradation with/without enzyme (Collagenase 50ug/ml). (c) The compression modulus of the hydrogels. Comparing to PEG, G-1.5 and G-2.5 were softer and degraded faster during the incubation.*

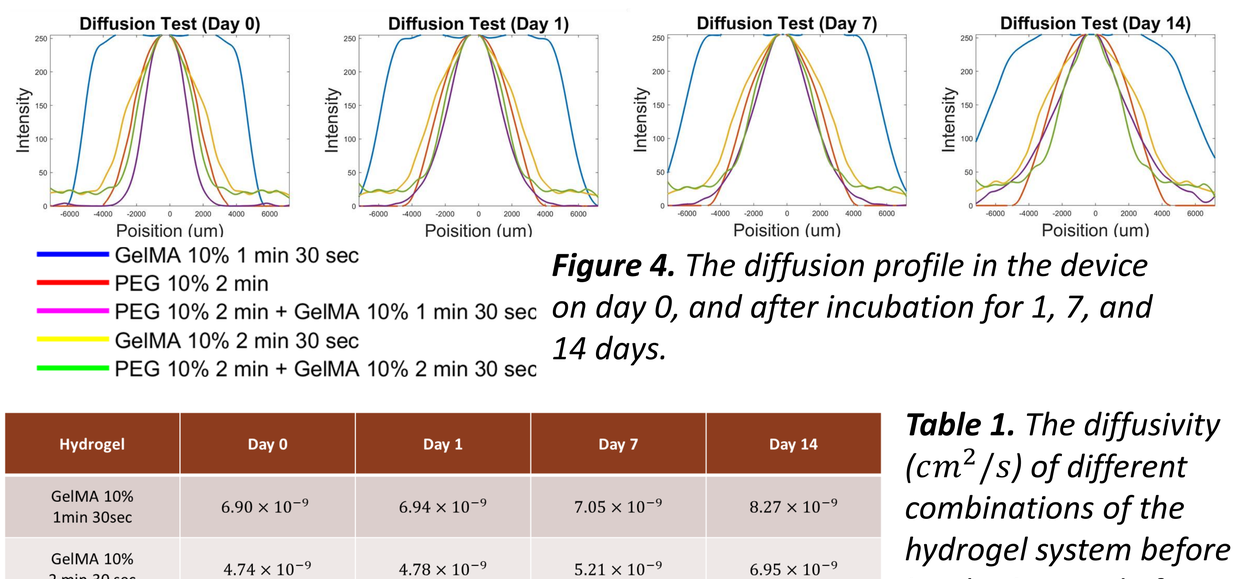

Figure 4. *The diffusion profile in the device on day 0, and after incubation for 1, 7, and 14 days.*

Hydrogel	Day 0	Day 1	Day 7	Day 14
GelMA 10% 1min 30sec	6.90×10^{-9}	6.94×10^{-9}	7.05×10^{-9}	8.27×10^{-9}
GelMA 10% 2 min 30 sec	4.74×10^{-9}	4.78×10^{-9}	5.21×10^{-9}	6.95×10^{-9}
PEG 10% 2 min	1.22×10^{-9}	1.31×10^{-9}	1.43×10^{-9}	2.03×10^{-9}
PEG 10% 2 min + GelMA 10% 1min 30sec	3.12×10^{-9}	3.25×10^{-9}	3.45×10^{-9}	5.21×10^{-9}
PEG 10% 2 min + GelMA 10% 2min 30sec	2.20×10^{-9}	2.25×10^{-9}	2.37×10^{-9}	3.28×10^{-9}

Table 1. *The diffusivity (cm^2/s) of different combinations of the hydrogel system before incubation, and after incubating for 1, 7 and 14 days. The diffusivity of G-1.5 is significantly different with other groups.*

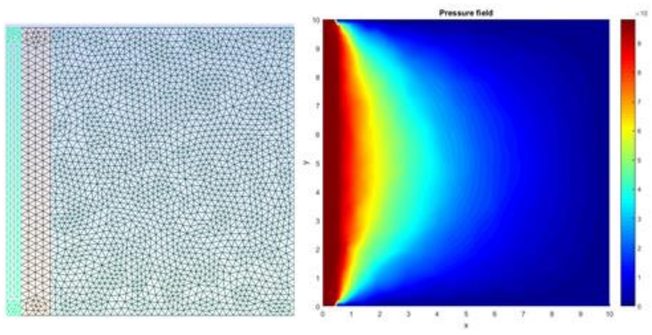

Figure 5. *The Finite Element Model of the dual-hydrogel system, and the corresponding diffusion profile based on the experimental diffusivity.*

CONCLUSION

The SEM images indicated both G-1.5 and G-2.5 had homogeneous pores and high porosity (>50%) compared with PEG based hydrogel. We prepared the photo-crosslinkable PEG-based hydrogel that was relatively stiff (E=~27 kPa) and slowly degradable enough to provide a constant flow rate through the channel. As for GelMA hydrogel, G-1.5 and G-2.5 were relatively soft, 1 kPa and 12 kPa, respectively. Both were fast degradable by enzymatic activity within 14 days. With this combination, PEG-based channel structure was maintained during the long time period in vitro culture, while microvascular networks were formed in GelMA (Data not shown). The diffusivities of the hydrogel system showed significant difference among different combination of hydrogel systems. Especially, the diffusivity of G-1.5 was significantly higher than other groups. However, the diffusivity of each system did not change significantly after incubation with PBS solution for 1, 7 and 14 days. Based on our diffusivity test, we built a finite element model to simulate the diffusion in the hydrogel system as shown figure 5. We will further study about the effect of shear stress induced by the luminal flow through the channel that will modulate endothelial functions and enhance formation of perfusable vascular networks.

ACKNOWLEDGMENTS

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